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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Schoenfeld *et al.*		
SERIAL NUMBER:	09/806,400	EXAMINER:	Ronald Schwadron
FILING DATE:	March 30, 2001	ART UNIT:	1644
FOR:	COMPOSITIONS FOR THE PREVENTION AND/OR TREATMENT OF ATHEROSCLEROSIS		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DROR HARATS UNDER 37 C.F.R. §1.132

I, Dror Harats, of 71 Mendes Street, 53 765 Ramat Gan, Israel, declare and state that:

1. I am a coinventor, together with Yahuda Shoenfeld and Jacob George, in the above-referenced patent application.

2. I received an M.D. degree from the Hebrew University Hadassah Medical School, Jerusalem, Israel. I worked as a post-doctoral fellow at the University of California at San Francisco from 1991-1994.

3. I am presently employed as the head of the "Institute of Lipids and Atherosclerosis" at the Sheba Medical Center in Tel-Hashomer, Israel. I am an Associate Professor of Medicine at the Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel. I also serve as the Secretary of the Israeli Society for Research, Prevention and Treatment of Atheroclerosis, and drafted the Guidelines for Prevention of Cardiovascular Diseases in Israel, and am a member in good standing of the European Taskforce for Prevention and Treatment of Atherosclerosis and Cardiovascular Diseases.

4. My research focuses on atherosclerosis. Since the beginning of my career, I have published over 80 scientific articles in highly regarded journals and books, and have presented my achievements at many international scientific conferences.

5. I have reviewed the Office Action dated June 24, 2003. I understand that claims 14, 18 and 20 are rejected under 35 U.S.C. §102(d) as anticipated by U.S. Patent No. 4,874,795 to Yesair ("Yesair").

6. I have reviewed the present application in conjunction with the Yesair reference.

7. Yesair teaches the use of lipid compositions for delivery and release of drugs and other substances via the lymphatics into the systemic circulation. Yesair describes a composition comprised of: (1) non-esterified fatty acids having 14-18 carbon atoms, (2) monoglycerides which are monoesters of glycerol and fatty acids having 14-18 carbon atoms, (3) lysophosphatidyl choline (LPC) in which the fatty acid component has 14-18 carbon atoms, and (4) a drug.

8. The present invention teaches an immunological oral tolerance-inducing composition consisting of oxidized low density lipoprotein (Ox-LDL) or malondialdehyde LDL (MDA-LDL) for the prevention or treatment of atherosclerosis. The present invention therefore eliminates the need for additional fatty acids or lipids when using Ox-LDL or MDA-LDL. Yesair does not teach or suggest the use of LPC, or any Ox-LDL, MDA-LDL or modified LDL, without the presence of other lipids

9. Moreover, in the experiments described in Appendix 1, Figure 1, esteric as well as etheric phospholipids, such as LPC (Lysolecitin) and an non-oxidized derivative (V) were shown to be ineffective in reducing atherogenesis in mice when compared to h-OxLDL (Human Oxidized LDL) or an oxidized derivative of OxLDL (CI-101), which each reduced atherogenesis by 50%.

10. I also understand that Claims 14, 18-20 and 26 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement.

11. I am aware that the Examiner has asserted that the LDL receptor (LDLR) deficient mouse model (as described in the Specification at, e.g., page 15, lines 20-29; and page 18, line 18 to page 19, line 31) is not a working example under the *Wands* test, since in other diseases (such as multiple sclerosis and rheumatoid arthritis), treatments that were effective in mice were not effective in humans. It is my position that the LDLR deficient mice used in the

studies disclosed in the present application is the preferred, art-recognized model of the biochemical and morphologic effects of atherosclerosis, and is thus a working example under the *Wands* test.

Specifically, mice having targeted inactivation of the apolipoprotein E (ApoE) gene and of the LDLR gene, under appropriate conditions, develop complex atherosclerotic lesions and provide practical atherosclerotic mouse models and are the most utilized model to study lipids and atherosclerosis. ApoE is critical in lipoprotein trafficking (clearance of chylomicrons, VLDL, and HDL). Thus mice lacking apoE have plasma cholesterol levels that are 4 to 5 times normal and develop atherosclerotic lesions spontaneously, even when fed a normal diet. The lesions resemble human lesions and progress over time from an initial fatty streak to a complex lesion with a fibrous cap. Mice lacking the LDLR have less overt disease, with a modest 2 times normal plasma cholesterol level when maintained on a normal diet, and they develop atherosclerosis only slowly. However, in response to a high-fat, high-cholesterol diet, LDLR-deficient mice exhibit massive elevations in plasma cholesterol and rapidly develop atherosclerotic lesions throughout the aorta.

The predictable development of atherosclerotic lesions and plaques and there resemblance to human atherosclerotic lesions and plaques along with other more general advantages of mice, such as their small size, short generation time, and relative ease to care, have made the mouse a most valid, effective and practical model for the study of atherosclerosis

12. Furthermore, in utilizing the art-recognized LDLR deficient mouse model to study atherosclerotic lesions and plaques, several biochemical events and molecules (i.e. T-lymphocytes) are assessed to determine the extent of atherosclerotic lesion and plaque formation. Endothelial activation by oxidized lipoproteins initiates atherosclerotic lesions in humans via increased adhesion of mononuclear cells and their recruitment into the vascular wall. The recruited inflammatory cells produce and induce the expression of numerous inflammatory cytokines and chemokines, thus promoting lesion progression and determining plaque composition. T_H1 type T-lymphocytes (pro-inflammatory) produce cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-2, which activate macrophages. In contrast, T_H2 type T-lymphocyte (anti-inflammatory) produce TNF-β, IL-4,

IL-5, IL-10, and IL-13, which inhibit several macrophage functions. IL-12 and IFN-γ are found in atherosclerotic plaques both in humans and in atherosclerotic mice, demonstrating that atherosclerotic lesions contain T_H1-promoting cytokines. IFN-γ has been detected intracellularly and in lesions in areas surrounding T lymphocytes, and IFN-γ-producing T lymphocytes have been cloned from human atherosclerotic lesions. It is my position that the modulation of the T_H1/T_H2 balance is an effective treatment of atherosclerosis.

The spontaneous proliferation of human peripheral blood mononuclear cells (PBMC) towards antigens involved in atherogenesis (*e.g.*, oxidized LDL and MDA-oxidized LDL) was determined (*See*, Appendix 1, Figure 2). Figure 2 shows the stimulation index of human PBMC from donors suffering from CAD (coronary artery disease) is increased five-fold as compared to healthy donors. The proliferative response of PBMC in response to oxidized LDL and MDA-oxidized LDL, seen in patients suffering from coronary artery disease, demonstrates that the suppression of this response by induction of oral tolerance is a useful therapeutic treatment of atherosclerosis.

13. To orally formulate OxLDL for atherosclerosis treatment, LDL (1.019-1.063 g/ml) was isolated from sera of normal blood donors by density gradient ultracentrifugation. The human or murine native LDL was dialysed against PBS (pH 7.4) to remove EDTA. Protein concentration was determined using the Lowry method. Oxidation of LDL was performed by incubation 1mg of LDL protein/mL with 15μM CuSO4 for over night (16-20 hours) at 37° C. The extent of oxidation of the lipoprotein preparations was determined by thiobarbituric acid-reactive substance (TBARS) assay. The oxidized LDL was filtered through a 0.45μm syringe filter. Protein concentration was determined again using the Lowry method. Storage of OxLDL is at 2-8° C for no more than three months. For administration of OxLDL to mice the OxLDL is further diluted to the desired concentration in sterile PBS. Each mouse receives by gavages 200μl using a 20-gouge stainless steel animal-feeding needle, or 10μl intranasal by pipette.

14. Moreover, while reducing the present invention to practice, we orally administered OxLDL to mice according to the teachings of the instant specification and evaluated the aortic sinus lesion area and the cytokine expression levels in the aorta. As shown in Figure 1, oral

administration of OxLDL decreases the aortic sinus lesion area by 50% as compared to non treated mice. Furthermore OxLDL treatment induced an classic anti-inflammatory response as shown by the increased mRNA levels of IL-10 and decreased mRNA levels of IFN-γ in aortas as compared to the control (Appendix 1, Figure 3). Also, when mice were treated with an oxidized derivative of OxLDL, the inflammatory pattern results were similar to that shown with OxLDL (Appendix 1, Figure 4).

I assert that the present invention and these continuing studies show that oral administration of OxLDL or an oxidized derivative, such as MDA-LDL, following the teachings of the instant specification, can decrease atherogenesis and therefore by utilized to treat or prevent atherosclerosis in a subject. The present invention and these continuing studies also show this effect may be induced by immunological switch between pro-inflammatory response to an anti-inflammatory response including suppression of IFN-γ and that a decrease in IFN-γ expression can be used as a marker for atherosclerosis treatment with OxLDL or an oxidized derivative.

15. I further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

D. Harats

Dror Harats

Signed this 18 day of December, 2003

Encl:
Appendix I

TRA 1855303v2